

担子菌地星菌丝体中一个新的甾醇酯*

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摘要: 从担子菌地星 (*Astraeus hygrometricus*) 发酵培养菌丝体中分离得到一个新的多羟基甾醇酯, 其化学结构通过波谱学方法包括二维核磁共振鉴定为: 3, 5 -二羟基-(22*E*, 24*R*)-麦角甾醇-7, 22-二烯-6 -棕榈酸酯。同时还从该菌中分离得到其它三个甾醇类化合物。

关键词: 地星; 甾醇酯; 担子菌

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A New Steryl Ester from the Culture Mycelia of the Basidiomycete *Astraeus hygrometricus* (Astraceae)

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Abstract: A new steryl ester with a polyhydroxylated ergostane-type nucleus, 3, 5 -dihydroxy-(22*E*, 24*R*)-ergosta-7, 22-dien-6 -yl palmitate (**1**), together with three known compounds (**2** - **4**) was isolated from the culture mycelia of the basidiomycete *Astraeus hygrometricus*. The structure of compound **1** was elucidated on the basis of extensive spectroscopic methods (IR, HR-FAB-MS, 1D and 2D NMR).

Key words: *Astraeus hygrometricus*; Steryl ester; Basidiomycete

Astraeus hygrometricus (Sclerodermatales, Basidiomycete), a mycorrhizal fungus, is widely distributed in China. It is also used as a remedy for haemastatic and inflammation in traditional Chinese medicine (Mao, 2000). Three triterpenes and a splenocyte activity glucan have previously been isolated from the fruiting bodies of this fungus (Takaishi *et al.* 1987; Chakraborty *et al.* 2004). To the best of our knowledge, there are no chemical investigations on the culture broth of this fungus. In our continuing studies on the basidiomycete-derived secondary metabolites (Liu, 2002, 2005, 2006; Shao *et al.* 2005; Wang and Liu,

2005), we have been isolated a new steryl ester with a polyhydroxylated ergostane-type nucleus, 3, 5 -dihydroxy-(22*E*, 24*R*)-ergosta-7, 22-dien-6 -yl palmitate (**1**) (Fig. 1), as well as three known compounds (22*E*, 24*R*)-5, 8 -epidioxyergosta-3, 22-dien-3 -ol (**2**), (22*E*, 24*R*)-ergosta-4, 6, 8 (14), 22-tetraen-3 -one (**3**), and (22*E*, 24*R*)-ergosta-7, 22-dien-3 -ol (**4**) from the culture mycelia of the fungus. It is noted that there are few naturally occurring polyhydroxylated steryl esters reported up to date (Wang and Liu, 2005; Yang *et al.* 2003; Zhang *et al.* 2005). This paper deals with the isolation and struc-

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ture elucidation of the new steryl ester (1) .

Results and Discussion

Compound 1 was obtained as a colorless oily solid . The molecular formula was determined to be C₄₄ H₇₆ O₄ by ¹³ C-NMR data and HR-FAB-MS (calc . for [M + H]⁺ : 669.5821; found: 669.5814) . Its IR spectrum revealed the presence of hydroxyl groups (3 431 cm⁻¹) . The ¹ H-NMR spectrum (Table 1) which assigned with aid of the ¹ H-¹ H COSY spectrum (Fig . 2), exhibited two tertiary methyl signals at 1.01 (s, H - 19) and 0.56 (s, H - 18), four secondary methyl signals at 1.02 (d, J = 6.6, H - 21), 0.91 (d, J = 6.9, H - 28), 0.83 (d, J = 7.2, H - 27) and 0.82 (d, J = 7.2, H - 26), a pair of 1, 2-disubstituted olefinic protons at 5.22 (dd, J = 15.2, 7.4, H - 23) and 5.15 (dd, J = 15.2, 8.0, H - 22), and a trisubstituted olefinic proton at 4.88 (s, H - 7) . Its ¹³ C-NMR analyzed together with the DEPT and HMQC spectra also showed signals due to an oxygenated quaternary carbon at 75.2 (s, C - 5), two oxygenated methane carbons at 73.7 (d, C - 6) and 67.3 (d, C - 3), and four olefinic carbons at 144.2 (s, C - 8), 135.4 (d, C - 22), 132.2 (d, C - 23) and 115.2 (d, C - 7) . Based on these data, 1 was suggested to be an ergosta-7, 22-dien-3 , 5 , 6-triol derivatives . Meanwhile in ¹ H-NMR spectrum, a characteristic downfield signal of H - 6 (5.27, s) caused by acylation effect distinctly indicated that the palmitate moiety was located at 6 position of the sterol nucleus . This was also supported by a cross peak observed between H

- 19 and H - 6 in the ROSEY spectrum . These data were in good agreement with those reported for ergosta-7, 22-dien-3 , 5 , 6 -triol and its derivatives (Ayer *et al* . 1992; Chen *et al* . 1991; Goldstein and Frye, 1996) . Furthermore, the ¹ H- and ¹³ C-NMR of compound 1 revealed a terminal methyl signal at (0.88 (t, J = 6.8, H - 16)/14.1 (q, C - 6)), a methylene group in -position to an ester function at (2.30 (t, J = 7.6, H - 2)/34.6 (t, C - 2)), a carbonyl carbon at 173.4 (s, C - 1), and other signals at (1.64 (m, H - 3), 1.25 - 1.34 (overlapped), and 31.4 (t, C - 14), 25.1 (t, C - 3), 22.7 (t, C - 15), 29.2 - 29.8 (t)), which showed to be a saturated long-chain fatty-acid ester moiety . By comparison with the data in literature (Zhang *et al* . 2005), those data indicated that the saturated long-chain fatty-acid was palmitate . It was also proved by the EI-MS data, which displayed fragment ion peaks at m/z 412 (67 [M-C₁₆ H₃₂ O₂]⁺), 394 (34 [M-C₁₆ H₃₂ O₂ -H₂ O]⁺) and 376 (27 [M-C₁₆ H₃₂ O₂ -2H₂ O]⁺) . The linked position of the palmitate moiety was further confirmed by the clearly correlation between H - 6 (5.27, s) and C - 1 (173.4, s) in the HMBC spectrum (Fig . 2) . The geometry of the ²²-double bond was determined to be E from the coupling constant (J = 15.2) between H - 22 and H - 23 . The stereochemistry at C - 20 and C - 24 was deduced to be R and R, respectively, by comparison of ¹ H- and ¹³ C-NMR data with those of ergosterol (Wright *et al* . 1978) . From all above data, the structure of 1 was assigned as 3 , 5 -dihydroxy - (22 E, 24 R)-ergosta-7, 22- dien-6 -yl palmitate .

Table 1 ¹ H- and ¹³ C-NMR data of Compound 1 (CDCl₃) .

Position	C	H	Position	C	H
1	31.9 (t)	1.25 (m)	19	17.9 (q)	1.01 (s)
2	30.8 (t)	1.85 (m)	20	40.3 (d)	2.02 (m)
3	67.3 (d)	4.00 (m)	21	21.1 (q)	1.02 (d, J = 6.6)
4	39.2 (t)	2.04 (m), 1.92 (m)	22	135.4 (d)	5.15 (dd, J = 15.2, 8.0)
5	75.2 (s)	-	23	132.2 (d)	5.22 (dd, J = 15.2, 7.4)
6	73.7 (d)	5.27 (s)	24	42.9 (d)	1.85 (m)
7	115.2 (d)	4.88 (s)	25	33.1 (d)	1.47 (m)
8	144.2 (s)	-	26	19.6 (q)	0.82 (d, J = 7.2)
9	43.5 (d)	2.09 (m)	27	19.9 (q)	0.83 (d, J = 7.2)
10	39.0 (s)	-	28	17.6 (q)	0.91 (d, J = 6.9)
11	21.3 (t)	1.57 (m)	1	173.4 (s)	-
12	39.6 (t)	1.52 (m), 1.30 (m)	2	34.6 (t)	2.36 (t, J = 7.6)
13	43.8 (s)	-	3	25.1 (t)	1.64 (m)
14	54.8 (d)	1.90 (m)	4 - 13	29.2 - 29.8 (t)	1.25 - 1.34 (m)
15	22.7 (t)	1.40 (m)	14	31.4 (t)	1.25 - 1.34 (m)
16	28.0 (t)	1.73 (m)	15	22.7 (t)	1.25 - 1.34 (m)
17	55.9 (d)	1.28 (m)	16	14.1 (q)	0.88 (t, J = 6.8)
18	12.2 (q)	0.56 (s)			

Assignment made on the basis of ¹ H-¹ H COSY, HMQC and HMBC data .

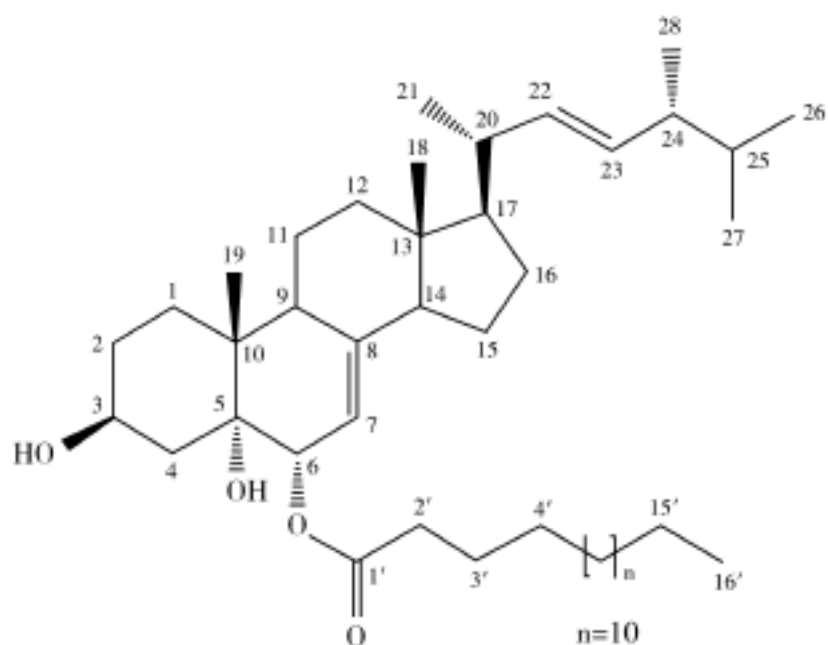
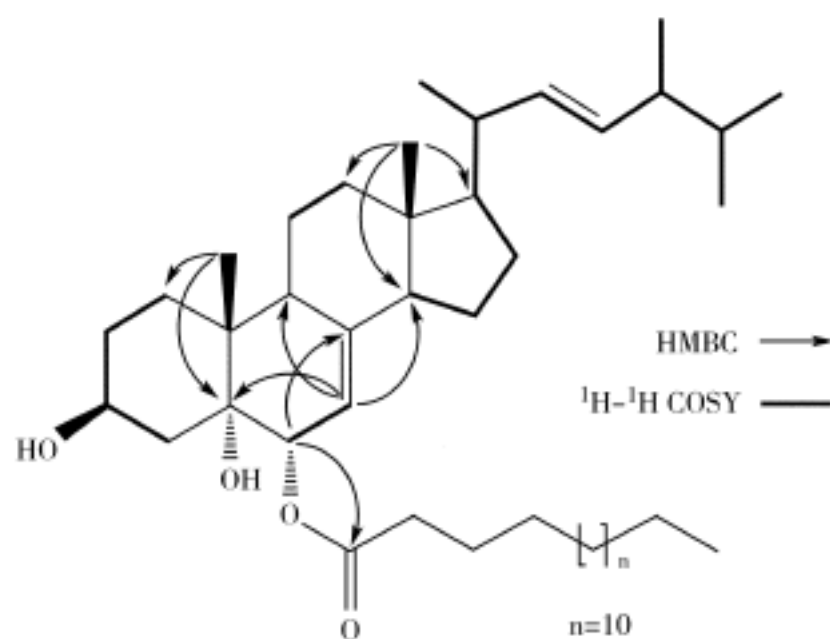


Fig. 1 The structure of compound 1

Fig. 2 ^1H - ^1H COSY and key HMBC correlations of compound 1

Compounds **2**, **3**, and **4** were also isolated from the same fungus, and identified as (22*E*, 24*R*)-5, 8-epidioxyergosta-3, 22-dien-3-ol (**2**), (22*E*, 24*R*)-ergosta-4, 6, 8 (14), 22-tetraen-3-one (**3**), and (22*E*, 24*R*)-ergosta-7, 22-dien-3-ol (**4**), respectively, according to the physical and spectroscopic data in literatures (Yue *et al.* 2001; Gao *et al.* 2001; Lu *et al.* 1985).

Experimental

General Optical rotation was measured on a Horiba SE-PA-300 spectropolarimeter. IR spectrum was obtained with a Bruker Tensor 27 spectrometer, with KBr pellets, in cm^{-1} . 1D and 2D-NMR spectra were recorded on Bruker AV-400 and DRX-500 spectrometers in CDCl_3 , in ppm, J in Hz. EI-MS was recorded with a Thermo Finnigan Trace DSQ spectrometer, HR-FAB-MS was recorded with a VG Autospec-3000 spectrometer.

Silica gel (200-300 mesh, Qingdao Marine Chemical Inc, Qingdao, P. R. China), and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography. Pre-coated silica gel GF₂₅₄ plates (Qingdao Marine Chemical Inc, Qingdao, P. R. China) were used for TLC. Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH.

Mushroom Material and Culture The fungus *A. hygrometricus* was collected at the Botanic Garden of Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China, in September 2001, and identified by Dr. Fu-Qiang Yu, Kunming Institute of Botany. The voucher specimen was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences. Culture medium: potato (peeled) 200 g, glucose 20 g, KH_2PO_4 3 g, MgSO_4 1.5 g, citric acid 0.1 g and thiamin hydrochloride 10 mg in 1 L of deionized H_2O (pH 6.5 before autoclaving). The culture liquid was fermented at 25 °C for 20 days on a rotary shaker (150 r/min).

Extraction and Isolation The dried mycelia (106 g) filtered from culture broth (25 L) were successively extracted with $\text{CHCl}_3/\text{MeOH}$ (1:1). The extract was evaporated in vacuo and the oily residue (8.0 g) was subjected to CC (silica gel, petroleum ether/ Me_2CO (9:1:1)). The fraction (0.36 g) from petroleum ether/ Me_2CO (20:1) was further purified by CC (silica gel, petroleum ether/ AcOEt 20:1; Sephadex LH-20, $\text{CHCl}_3/\text{MeOH}$ 1:1) to afford the pure compound **3** (10 mg) and **4** (13 mg). The fraction (0.51 g) from petroleum ether/ Me_2CO (10:1) was further isolated by CC (silica gel, $\text{CHCl}_3/\text{AcOEt}$ 20:1) to give the pure compound **1** (6 mg) and **2** (20 mg).

Compound **1**, $\text{C}_{44}\text{H}_{76}\text{O}_4$, colorless oily solid; $[\alpha]_{\text{D}}^{24.4} = +46$ (c 0.17, CHCl_3); IR $_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 3431, 2956, 2925, 2852, 1710, 1629, 1461, 1381, 1187; ^1H - and ^{13}C -NMR data: see Table 1; EI-MS: 412 (67 $[\text{M}-\text{C}_{16}\text{H}_{32}\text{O}_2]^+$), 394 (34 $[\text{M}-\text{C}_{16}\text{H}_{32}\text{O}_2-\text{H}_2\text{O}]^+$), 376 (27 $[\text{M}-\text{C}_{16}\text{H}_{32}\text{O}_2-2\text{H}_2\text{O}]^+$), 251 (100), 157 (48), 129 (30), 109 (15), 69 (56); HR-FAB-MS (pos.): 669.5814 ($[\text{M}+\text{H}]^+$, $\text{C}_{44}\text{H}_{77}\text{O}_4^+$, calc. 669.5821).

References:

- Ayer WA, Ma YT, 1992. Sirosterol and dehydroazasirosterol, unusual steroidal adducts from a *Sirococcus* species [J]. *Can J Chem*, **70**: 1905-1913.
- Chakraborty I, Mondal S, Pramanik M, 2004. Structural investigation of a water-soluble glucan from an edible mushroom *Astraeus hygrometricus* [J]. *Carbohydr Res*, **399**: 2249-2254.
- Chen RY, Wang YH, Yu DQ, 1991. Studies on the chemical constituents of the spores from *Ganoderma lucidum* [J]. *Acta Bot Sin*, **33**: 65-68.
- Gao JM, Dong ZJ, Liu JK, 2001. A new ceramide from the basidiomycete *Russula cyanoxantha* [J]. *Lipids*, **36**: 175-180.
- Goldstein AS, Frye LL, 1996. Synthesis and bioevaluation of delta 7-5-desaturase inhibitors, an enzyme late in the biosynthesis of the fungal sterol ergosterol [J]. *J Med Chem*, **39**: 5092-5099.
- Liu JK, 2002. Biologically active substances from mushrooms in Yunnan, China [J]. *Heterocycles*, **57**: 157-167.
- Liu JK, 2005. N-containing compounds of macromycetes [J]. *Chem*

Rev, **105**: 2723 2744

Liu JK, 2006 . Natural terphenyls: developments since 1877 [J] . *Chem Rev*, **106**: 2209 2223

Lu W, Adachi I, Kano K *et al* . 1985 . Platelet aggregation potentiators from cho-rei [J] . *Chem Pharm Bull*, **33**: 5083 5087

Mao XL (卯晓岚), 2000 . The Macrofungai in China (中国大型真菌) [M] . Zhengzhou: Henan Sciences & Technology Press, 522

Shao HJ, Qing C, Wang F *et al* . 2005 . A new cytotoxic lanostane triterpenoid from the basidiomycete *Hebeloma versipelle* [J] . *J Antibiot*, **58**: 828 831

Takaishi Y, Murakami Y, Ohashi T *et al* . 1987 . Three triterpenes from *Astraeus hygrometricus* [J] . *Phytochemistry*, **8**: 2341 2344

WangF, Liu JK, 2005 . Two new steryl esters from the basidiomycete *Tricholomopsis rutilans* [J] . *Steroids*, **70**: 127 130

Wright JLC, Mcinnes AG, Shimizu S *et al* . 1978 . Identification of C-24 alkyl epimers of marine sterols by ¹³C nuclear magnetic resonance spectroscopy [J] . *Can J Chem*, **56**: 1898 1903

Yang SP, Xu J, Yue JM, 2003 . Sterols from the fungus *Catathelasma imperiale* [J] . *Chin J Chem*, **10**: 161 165

Yue JM, Chen SN, Lin ZW *et al* . 2001 . Sterols from the fungus *Lactarium volenus* [J] . *Phytochemistry*, **56**: 801 806

Zhang P, Li X, Li N *et al* . 2005 . Antibacterial constituents from fruit bodies of ascomyce *Bulgaria inquinans* [J] . *Arch Pharm Res*, **28**: 889 891

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